

Cell death after anti-Wnt Ab incubation (%)

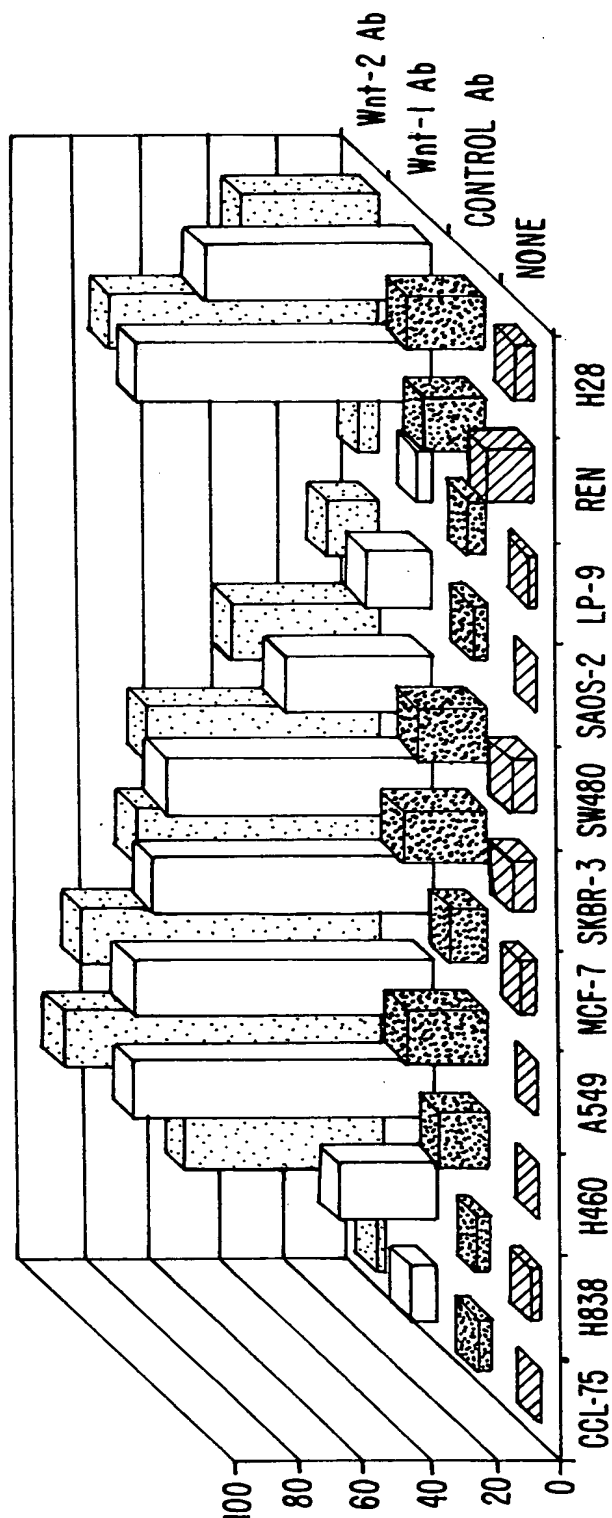
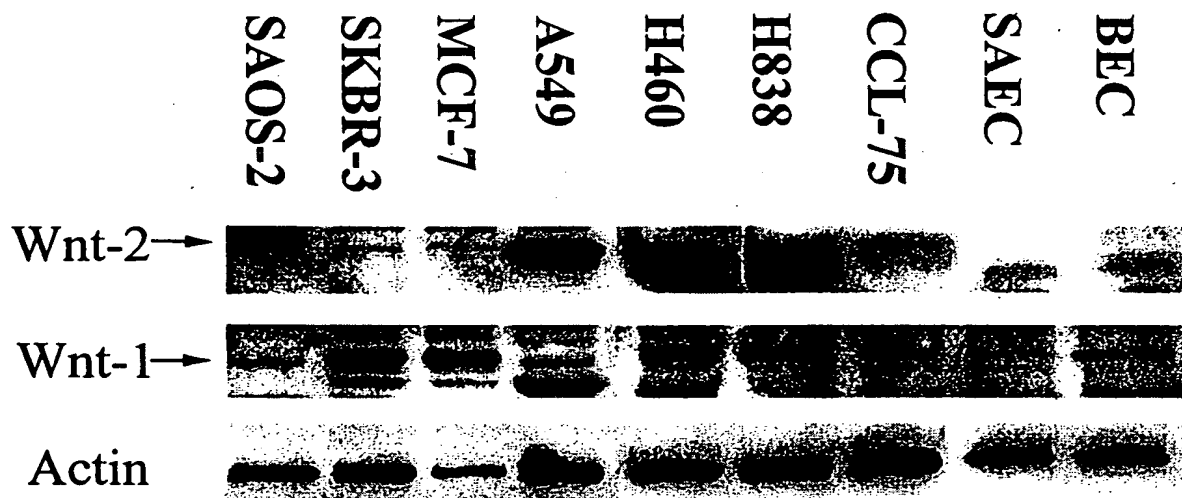


FIG. 1

**Fraction of apoptotic cell death (%) after anti-Wnt  
antibody treatment**

Cell Line	No Treatment	Control Ab	Anti-Wnt-1 Ab	Anti-Wnt-2 Ab
CCL-75 (normal lung)	0.9 ±0.3	3.8 ±2.3	5.8 ±2.1	2 ±1.1
H838 (lung cancer)	1.7 ±0.8	3.1 ±3.0	28.4 ±4.6	58.3 ±8.1
H460 (lung cancer)	0.6 ±0.3	12.7 ±7.0	83.8 ±5.6	90.7 ±6.5
A549 (lung cancer)	0.3 ±0.1	4.9 ±3.0	84.7 ±3.3	82.3 ±4.5
MCF-7 (breast cancer)	0.7 ±0.4	1.6 ±0.7	56.7 ±3.9	47.2 ±4.2
SKBR-3 (breast cancer)	0.4 ±0.1	5.8 ±1.3	67.9 ±6.1	56.4 ±4.8
SW-480 (colon cancer)	5.9 ±0.8	14.6 ±4.6	43.3 ±4.4	48.9 ±5.2
SAOS-2 (sarcoma)	1.1 ±0.4	4.2 ±1.6	20.0 ±3.2	16.5 ±1.0
LP-9 (normal mesothelial)	2.3 ±1.1	5.1 ±2.7	4.1 ±1.5	6.2 ±2.6
REN (mesothelioma)	13.6 ±2.4	15.2 ±6.5	89.6 ±3.8	81.4 ±8.4
H28 (mesothelioma)	6.6 ±3.5	12.4 ±7.4	68.7 ±5.8	41.3 ±6.6

**FIG. 2**



**FIG. 3A**

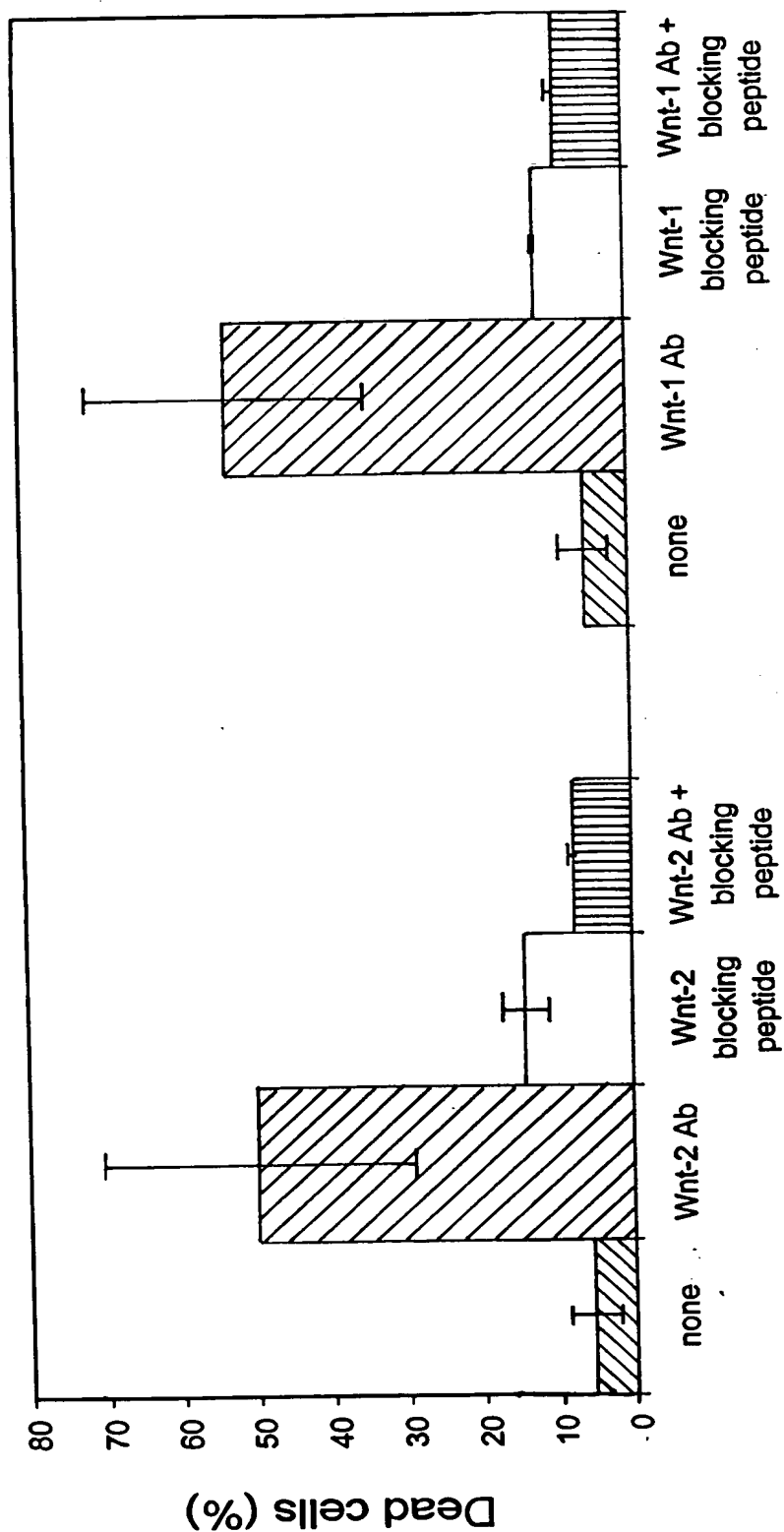


FIG. 3B

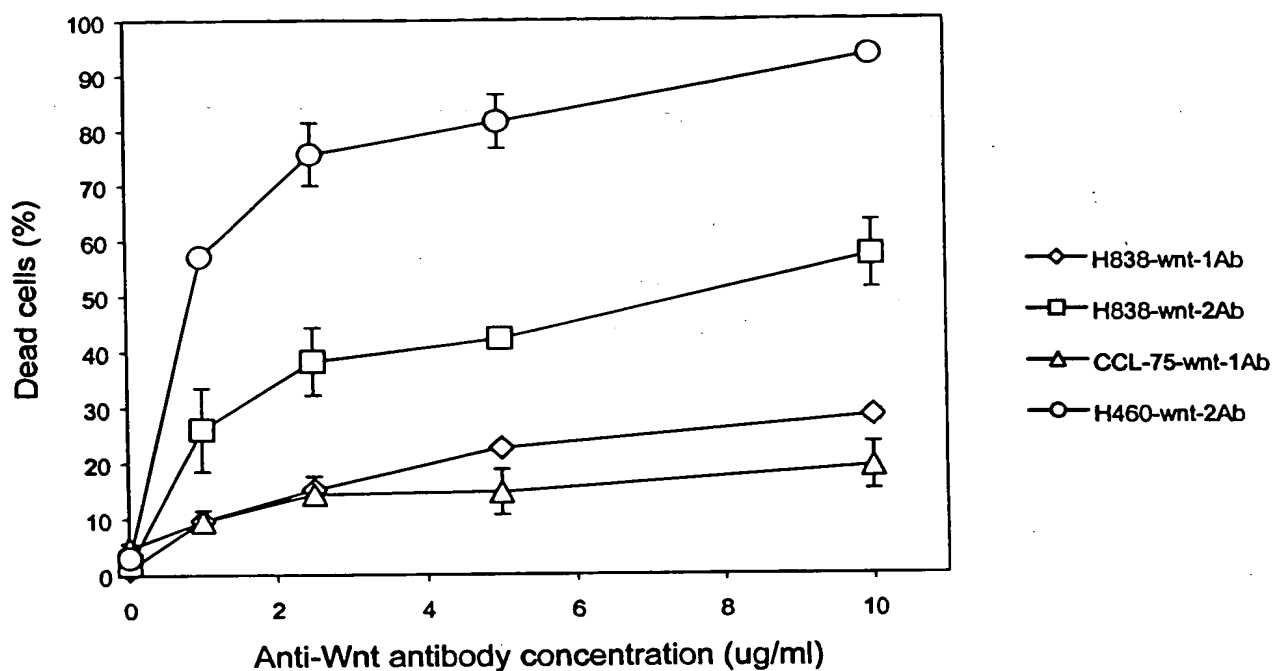
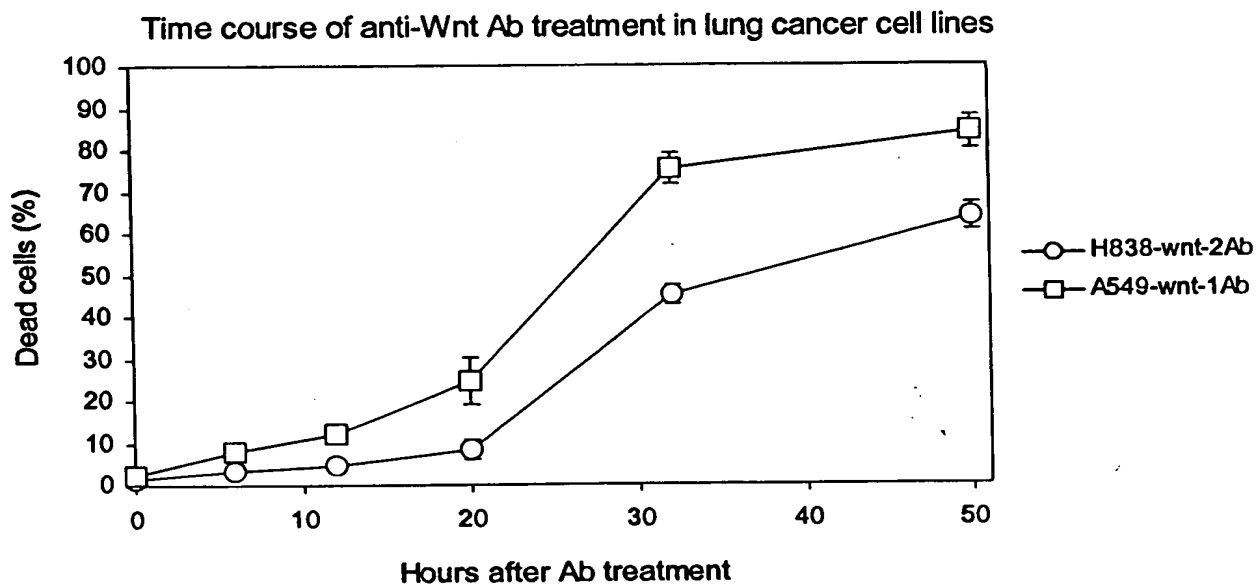
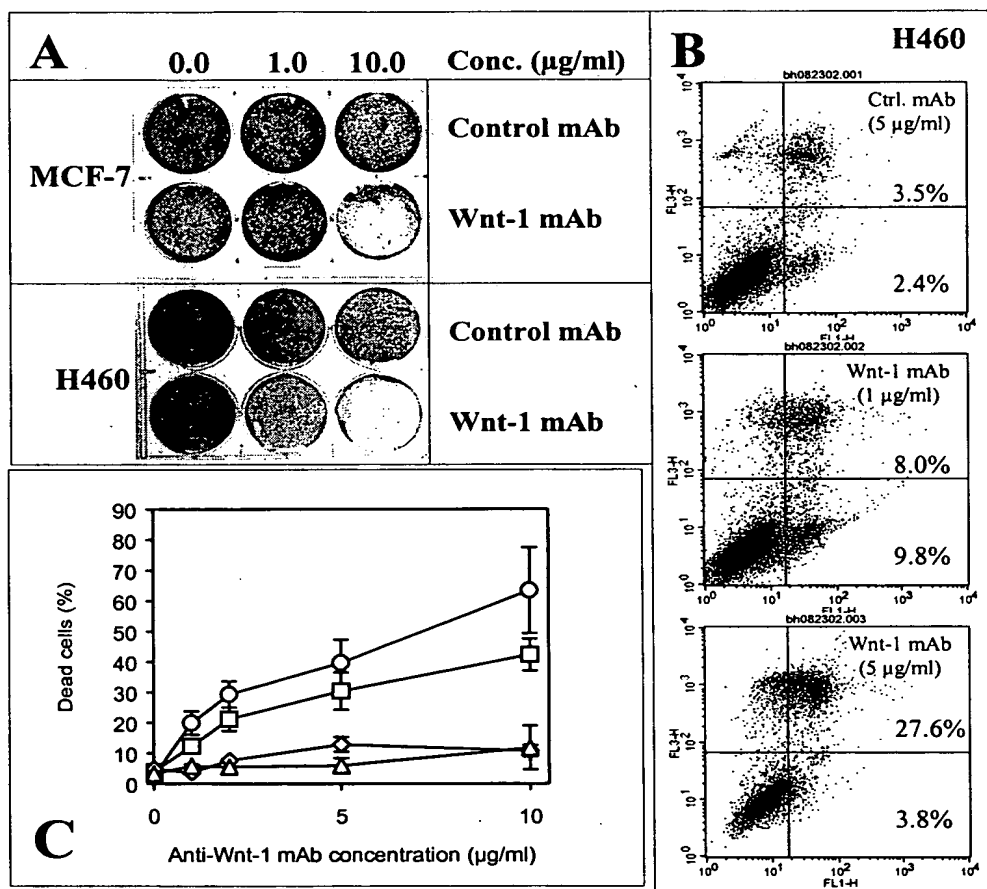
**FIG. 4A****FIG. 4B**

FIGURE 5



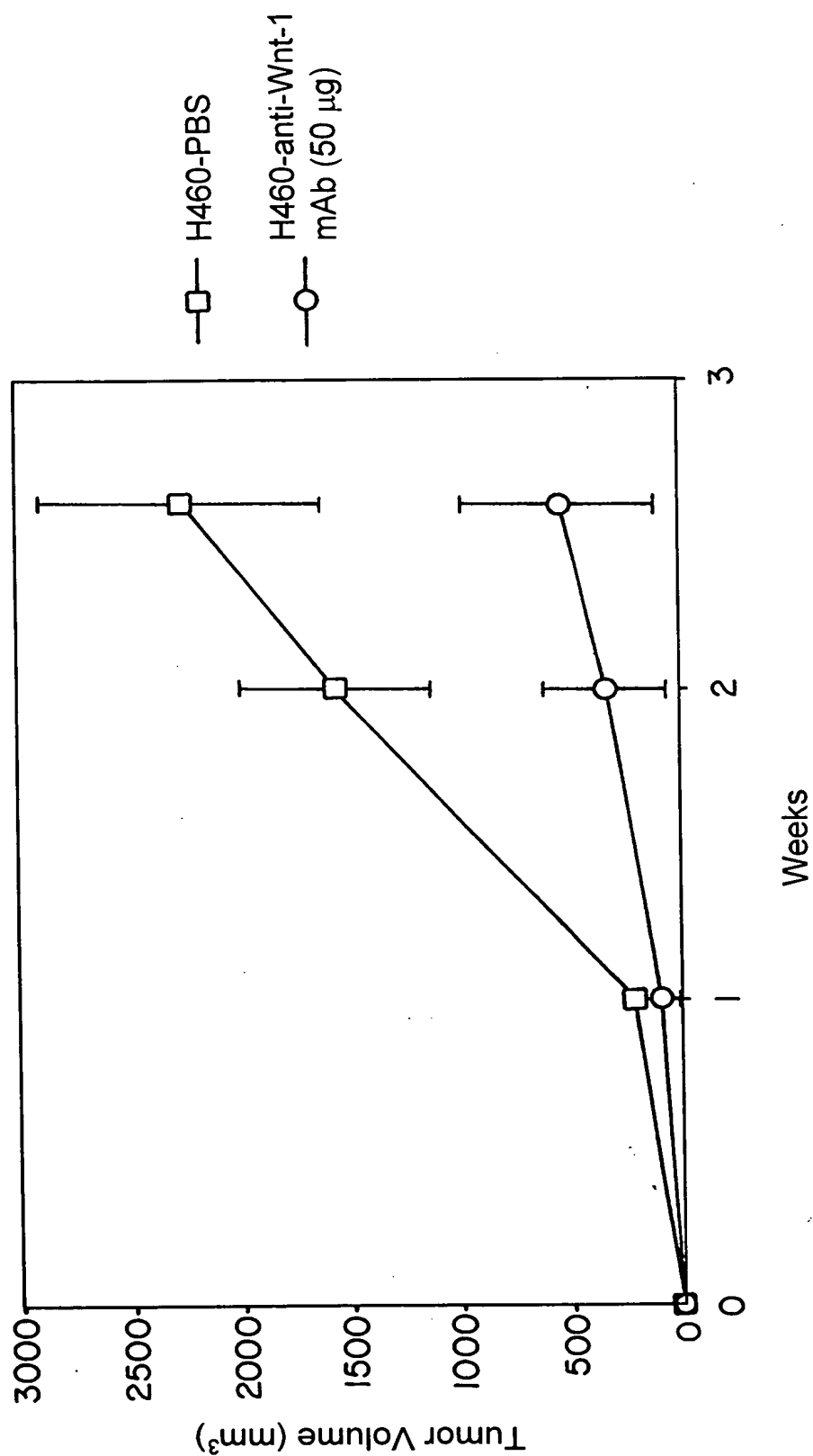


FIG. 6A

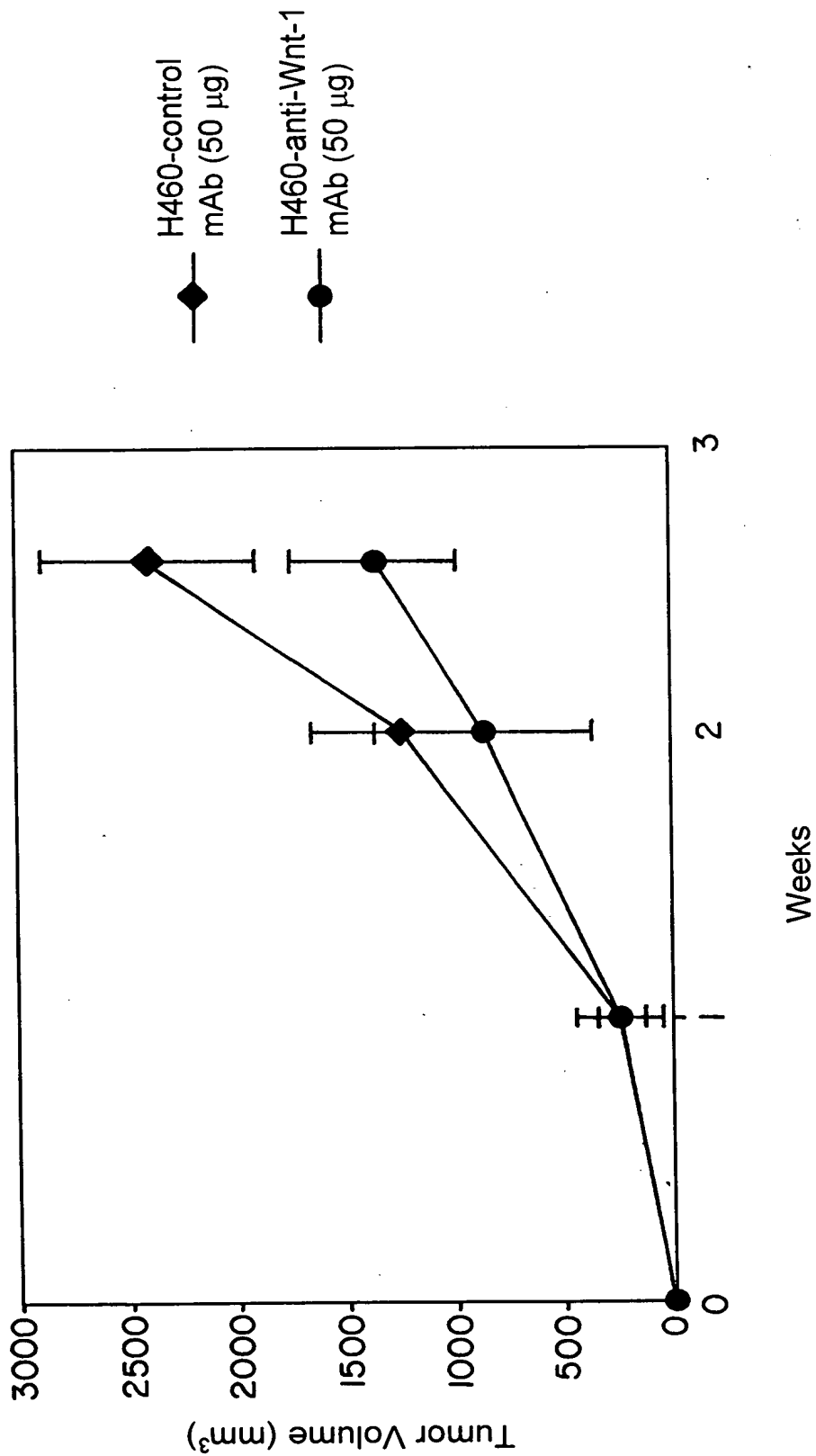
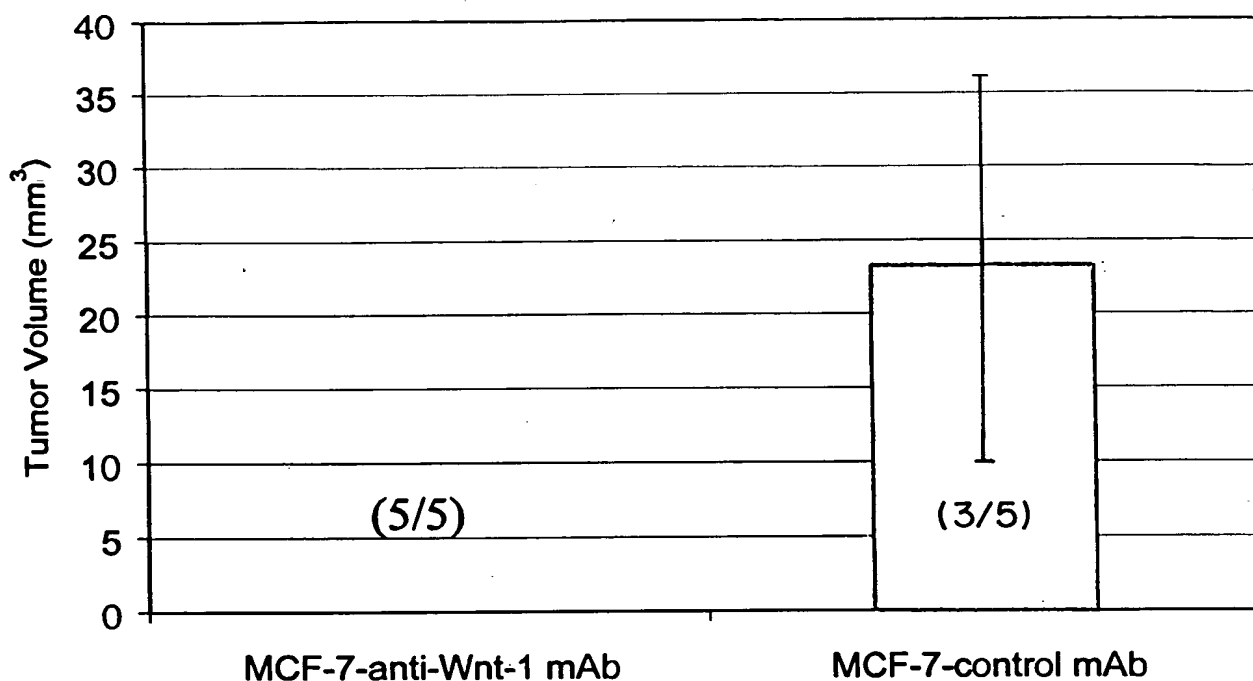


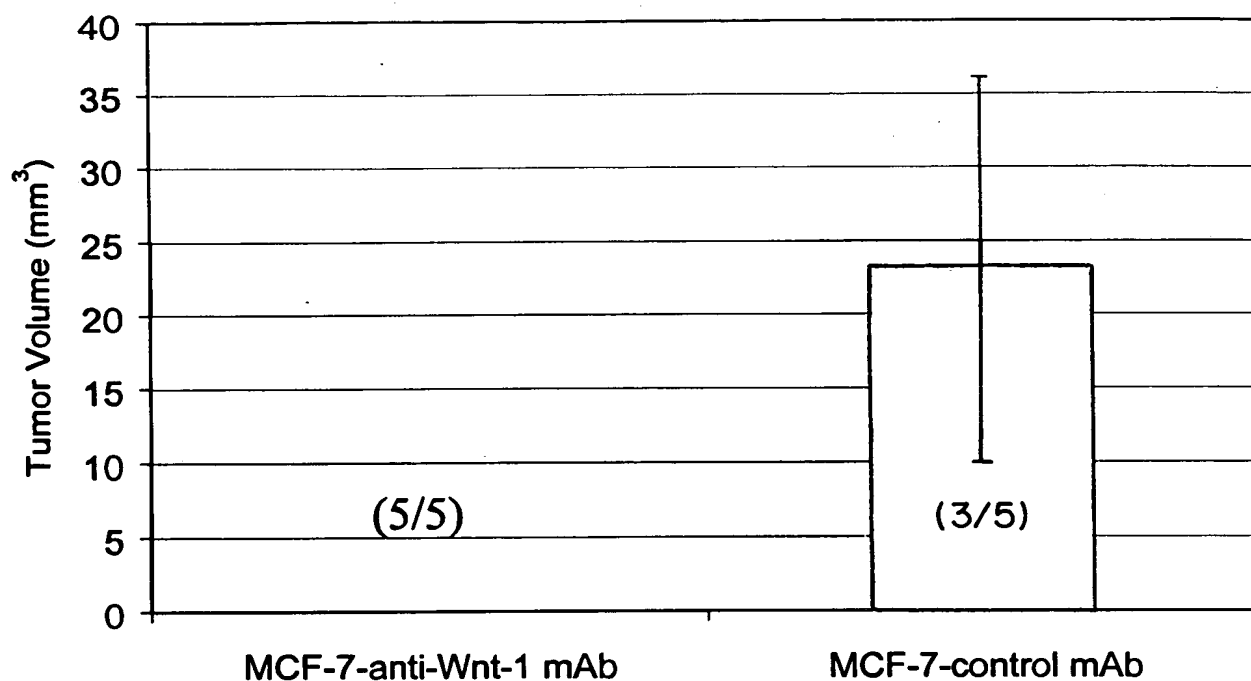
FIG. 6B





*in vivo* study on breast cancer. Tumor volume is shown after 3 weeks treatment with anti-Wnt-1 monoclonal antibody and control monoclonal antibody. 5 animals are in each group. None of the animals grows tumor after anti-Wnt-1 mAb injection, but three out of five animals grow tumor in the control group. (I.P. injection was done once weekly one week after inoculation of the MCF-7 cells)

**FIG. 6C**



*in vivo* study on breast cancer. Tumor volume is shown after 3 weeks treatment with anti-Wnt-1 monoclonal antibody and control monoclonal antibody. 5 animals are in each group. None of the animals grows tumor after anti-Wnt-1 mAb injection, but three out of five animals grow tumor in the control group. (I.P. injection was done once weekly one week after inoculation of the MCF-7 cells)

**FIG. 6C**

## Figure 7

1) CDR and FR region (light chain kappa) amino acid sequence of a anti-WNT1 antibody (Seq ID No:4)

Clone #1

<----- F R 1 - I M G T ----->

1y21

1 5 10 15 20  
D I V L T Q S P A S L A V S L G Q R A T I S  
GAC ATT GTG CTG ACA CAG TCT CCT GCT TCC TTA GCT GTA TCT CTG GGG CAG AGG GCC ACC ATC TCA

<----->

CDR1 - IMGT

25 30 35 40  
Y R A S K S V S T S G Y S Y M H W N Q Q  
TAC AGG GCC AGC AAA AGT GTC AGT ACA TCT GGC TAT AGT TAT ... ATG CAC TGG AAC CAA CAG

1y21

F R 2 - I M G T ----->

CDR2 - IMGT

55 60 65  
K P G Q P P R L L I Y L V S N  
AAA CCA GGA CAG CCA CCC AGA CTC CTC ATC TAT CTT GTA TCC ... AAC

1y21

----- F R 3 - I M G T -----

70 75 80 85  
L E S G V P A R F S G S G S G T D F T  
CTA GAA TCT GGG GTC CCT ... GCC AGG TTC AGT GGC AGT GGG ... TCT GGG ACA GAC TTC ACC

1y21

<----->

CDR3 - IMGT

90 95 100 105 110  
L N I H P V E E E D A A T Y Y C Q H I R E L  
CTC AAC ATC CAT CCT GTG GAG GAG GAG GAT GCT GCA ACC TAT TAC TGT CAG CAC ATT AGG GAG CTT

1y21  
115  
T R S E G G P S \* K N G  
ACA CGT TCG GAG GGG GGA CCA AGC TGA AAA AAC GGG

clone #2

<----- F R 1 - I M G T ----->  
1 5 10 15 20  
D I V V T Q S P A S L A V S L G Q R A T I S  
1y22W11krs GAC ATT GTG GTG ACA CAG TCT CCT GCT TTA GCT GTA TCT CTG GGG CAG AGG GCC ACC ATC TCA

-----> <----->  
CDR1 - IMGT  
25 30 35 40  
Y R A S K S V S T S G Y S Y M H W N Q Q  
1y22W11krs TAC AGG GCC AGC AGC AAA AGT GTC AGT ACA TCT GGC TAT AGT TAT ... ATG CAC TGG AAC CAA CAG

F R 2 - I M G T -----> <----->  
CDR2 - IMGT  
45 50 55 60 65  
K P G Q P P R L L I Y L V S N  
1y22W11krs AAA CCA GGA CAG CCA CCC AGA CTC ATC TAT CTT GTA TCC ... AAC

-----> <----->  
70 75 80 85  
L E S G V P A R F S G S G S G T D F T  
1y22W11krs CTA GAA TCT GGG GTC CCT ... GCC AGG TTC AGT GGC AGT GGG ... TCT GGG ACA GAC TTC ACC

----->

CDR3 - I

90 95 100 105 110

L N I H P V E E E D A A T Y Y C Q H I R E L

1y22w11krs CTC AAC ATC CAT CCT GTG GAG GAG GAT GCT GCA ACC TAT TAC TGT CAG CAC ATT AGG GAG CTT

MGT 115 120

S T X R R G E P S \* N K R

1y22w11krs AGC ACG TTX CGG AGG GGG GAG CCA AGC TGA AAT AAA CGG

2) CDR and FR region (light chain kappa) amino acid sequence of a anti-WNT2 antibody (Seq ID No:9)

<----- F R I - I M G T ----->

1 5 10 15 20

D I V L T Q S P A S L A V S L G Q R A T I S

1y23w21krs GAC ATT GTG CTG ACA CAG TCT CCT GCT TCC TTA GCT GTA TCT CTG GGG CAG AGG GCC ACC ATC TCA

-----> <----->

CDR1 - IMGT

25 30 35 40

Y R A S K S V S T S G Y S Y M H W N Q Q

1y23w21krs TAC AGG GCC AGC AAA AGT GTC AGT ACA TCT GGC TAT AGT TAT ... ATG CAC TGG AAC CAA CAG

F R 2 - I M G T ----->

CDR2 - IMGT

45 50 55 60 65

K P G Q P P R L L I Y L V S N

1y23w21krs AAA CCA GGA CAG CCA CCC AGA CTC CTC ATC TAT CTT GTA TCC ... AAC

----- F R 3 - I M G T ----->

70 75 80 85  
L E S R R S P A R F S G Q W C L V Y R  
1y23w21kRs CTA GAA TCT AGG AGG TCA ... CCT GCC AGG TTC AGT GGT CAG ... TGG TGT CTG GTG TAC AGA

----->  
90 95 100 105 110  
L H P Q T S M P V G G G C L Q P D Y X C S  
1y23w21kRs CTT CAC CCT CAG ACA TCC ATG CCT GTC GGA GGA GGA TGC CTG CAA CCT GAT TAT XTG TGC AGC

MGT 115 120 125  
T L G S L H V T E G G P S \* K N x  
1y23w21kRs ACA TTA GGG AGC TTA CAC GTT ACG GAG GGG GGA CCA AGC TGA AAA AAC GG

3) CDR and FR region (heavy chain IgG1) amino acid sequence of a anti-WNT1 antibody (Seq ID No:4)

XGTTXCAGCCTGXAGGAGTCXGGTGGA...GGATTGGTGCAGCCTAAAGGGTCATTGAAACTCTCATGTGCAGCCTCTG  
GATTCACCTTTTAATACCTACGCC.....ATGAACTGGGTCCGCCAGGCTCCAGGAAAGGGTTTGGAAATGGGT  
TGCTCGCATAAGAACTAGACGTTATAATTCTGCAACATATTATGCCGATTCTGTGAAA...GACAGGTTACCATCTCC  
AGAGATGATTACGGGGCATGCTCTATCTGCAAAATGAACNACTTGAAAACTGAGGACACAGCCATGTATTACTGTGTGA  
GGC

4) CDR and FR region (heavy chain IgG1) amino acid sequence of a anti-WNT2 antibody (Seq ID No:9)

<----- F R I - I M G T -----

1 5 10 15 20  
1y25W2HGRs ... .AG TCX GGA CCT ... GAG CTG GTG AAG CCT GGG GCT TCA GTG AAG ATG TCC

----->> <----->

CDR1 - IMGT  
25 30 35 40  
C K A S G Y T F T D Y V L S W V K Q  
1y25W2HGRs TGC AAG GCT TCT GGA TAC ACA TTC ACT GAC TAT GTT ... TTA AGC TGG GTG AAG CAG

F R 2 - I M G T -----> <----

CDR2 - IMGT  
50 55 60 65  
R T G Q G L E W I G E I Y P G Y G S T Y  
1y25W2HGRs AGA ACT GGA CAG GGC CTT GAG TGG ATT GGA GAG ATT TAT CCT GGA TAT GGT AGT ACT ... TAC

-----> F R 3 - I M G T -----

70 75 80 85  
Y N E K F K G K A T L T A D K S S N T A Y  
1y25W2HGRs TAC AAT GAG AAG TTC AAG ... GGC AAG GCC ACA CTG ACT GCT GAC AAA TCC TCC AAC ACA GCC TAC

-----> <-----> CDR3 - I

90 95 100 105 110  
M Q L S S L T S E D S A V Y F C A R W G D C  
1y25W2HGRs ATG CAG CTC AGC AGC CTG ACA TCT GAG GAC TCT GCG GTC TAT TTC TGT GCA AGA TGG GGG GAT TGC

MGT  
115 120 125  
F C L S G A K G x L V x C L C  
1y25W2HGRs TTT TGC TTA TCT GGG GCC AAG GGA XCT CTG GTC AXC TGT CTC TGC

FIGURE 8

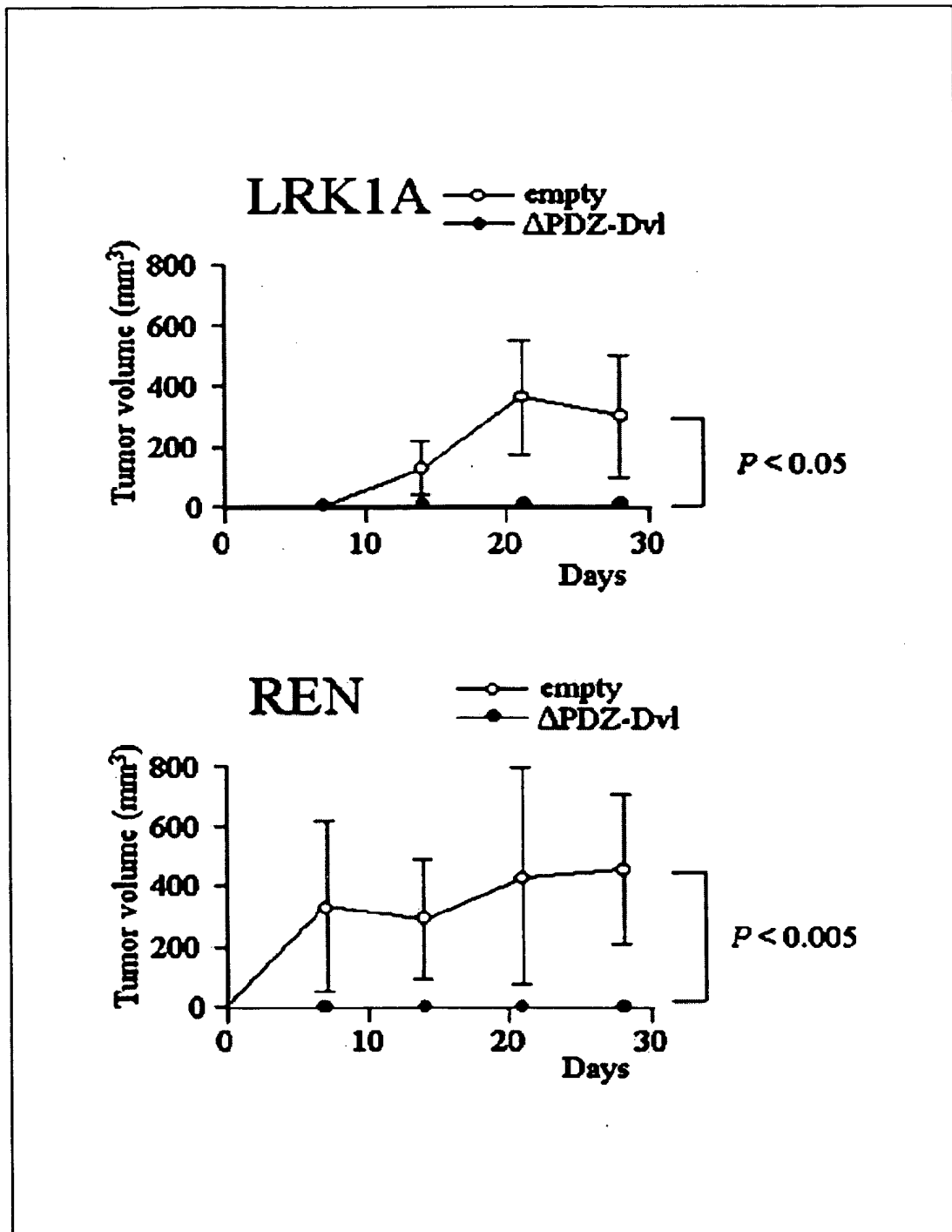




FIGURE 9

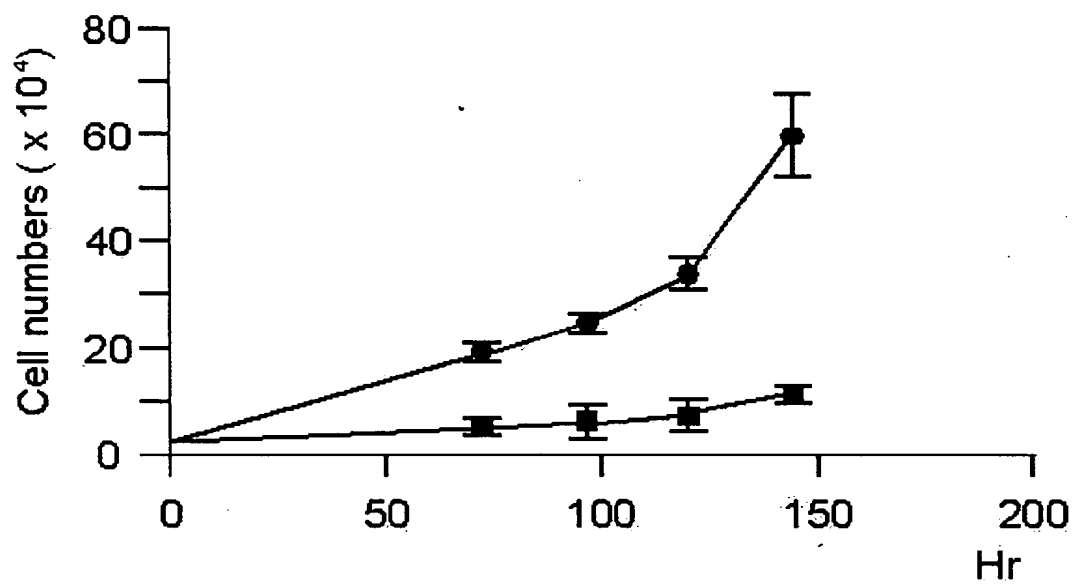


Figure 10. Over-expression of Wnt signal antagonist (FRP or DKK) induces apoptosis in cancer cell line MS-1

